

Technical note

Direct determination of Cu, Mn, Pb, and Zn in beer by thermospray
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Abstract

In this work, thermospray flame furnace atomic absorption spectrometry (TS-FF-AAS) was employed for Cu, Mn, Pb, and Zn determination in beer without any sample digestion. The system was optimized and calibration was based on the analyte addition technique. A sample volume of 300 μl was introduced into the hot Ni tube at a flow-rate of 0.4 ml min^{-1} using 0.14 mol l^{-1} nitric acid solution or air as carrier. Different Brazilian beers were directly analyzed after ultrasonic degasification. Results were compared with those obtained by graphite furnace atomic absorption spectrometry (GFAAS). The detection limits obtained for Cu, Mn, Pb, and Zn in aqueous solution were 2.2, 18, 1.6, and 0.9 $\mu\text{g l}^{-1}$, respectively. The relative standard deviations varied from 2.7% to 7.3% ($n=8$) for solutions containing the analytes in the 25–50 $\mu\text{g l}^{-1}$ range. The concentration ranges obtained for analytes in beer samples were: Cu: 38.0–155 $\mu\text{g l}^{-1}$; Mn: 110–348 $\mu\text{g l}^{-1}$; Pb: 13.0–32.9 $\mu\text{g l}^{-1}$, and Zn: 52.7–226 $\mu\text{g l}^{-1}$. Results obtained by TS-FF-AAS and GFAAS were in agreement at a 95% confidence level. The proposed method is fast and simple, since sample digestion is not required and sensitivity can be improved without using expensive devices. The TS-FF-AAS presented suitable sensitivity for determination of Cu, Mn, Pb, and Zn in the quality control of a brewery.

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1. Introduction

Beer has indeed become an international drink and some health benefits are associated with moderate consumption. Nowadays, the health effects of trace elements and the maximum concentration of trace elements in beers are controlled [1,2]. The determination of trace metals in beers

is relevant because they might be essential or toxic in the human body and they can also influence the brewing process. Moll [3] has listed numerous elements and compounds in the production water that can affect the quality of beer. Trace metals in beer may originate from natural sources (soil, water, cereal, hops, and yeast) as well as from environmental contamination due to fertilizers, pesticides, industrial processing, and containers [4]. Copper plays an important role as catalyst in the oxidation of organic compounds that are responsible for the stability and flavor of beers [5]. Manganese concentration influences the brewing process [6] and it can both activate and inhibit different enzymes [7]. Lead is a highly toxic element that accumulates in biological systems and has a long half-life.

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The source of lead in beer and in other alcoholic beverages could be the contamination of raw material and/or technological processes [8]. Zinc is known to be an essential nutrient, but it can be toxic depending on the concentration. Zinc constitutes about 33 mg kg⁻¹ of an adult body mass and it is essential as a constituent of many enzymes involved in several physiological functions, such as protein synthesis and energy metabolism [9].

An official method for beer analysis pointed out the need of destructing the organic matter before measurements by flame atomic absorption spectrometry (FAAS) [10]. This implied acid digestion with H₂SO₄ and H₂O₂ on a hot plate in an open beaker. This procedure is time-consuming and prone to losses and contamination [11].

However, the digestion of the sample can be avoided for the direct analysis of beer samples. Usually, direct aspiration of beer causes flame fluctuations and formation of solid deposits on the burner head. In general, only a few measurements can be carried out without clogging of the burner slot [12]. This problem could be minimized by higher dilution of beer samples; however, the typical sensitivities reached by FAAS do not allow the adoption of this strategy when determining minor and trace elements.

Thermospray flame furnace atomic absorption spectrometry (TS-FF-AAS) was recently proposed [13], and it makes possible complete sample introduction and also increases the sample residence time in the flame, thus improving the sensitivity. In the TS-FF-AAS system, a Ni tube is placed on a standard burner head of a FAAS spectrometer. The sample aerosol is introduced into the tube via a ceramic capillary that is heated only by the flame [14]. The end of the capillary reaches approximately 900 °C, which results in a very high temperature gradient along the capillary tip. The sensitivity for volatile elements is increased by up to one order of magnitude.

The application of TS-FF-AAS technique for direct analysis of slurry samples can help to improve two frequent difficulties in analytical procedures: the quantification of metals at trace levels and the time spent for sample preparation. Pereira-Filho et al. [15] investigated the use of TS-FF-AAS technique for determining Cd, Cu, and Pb in plant and animal tissues by slurry sample introduction. Simultaneous in situ sample digestion and analyte determination were performed in the thermospray flame furnace. Additionally, the feasibility of this technique for direct sample analysis was recently demonstrated for the determination of Cu and Zn in fruit juice and bovine milk samples [16].

In this work, TS-FF-AAS was employed for Cu, Mn, Pb, and Zn determination in beer without sample digestion. The adopted dilution factors were chosen depending on the analyte and sample characteristics to generate signals in the linear range of the calibration curve. The system was optimized and the analyte addition technique was employed for calibration.

2. Experimental

2.1. Instrumentation

A Varian SpectrAA-640 flame atomic absorption spectrometer (Varian, Mulgrave, Australia) equipped with a deuterium lamp background corrector was used. The instrumental parameters for Cu, Mn, Pb, and Zn determinations were set according to the manufacturer's recommendations (Table 1). For all analytes the air and acetylene flow-rates were kept at 13.5 l min⁻¹ and 2.0 l min⁻¹, respectively. A Varian Model SpectrAA-800 graphite furnace atomic absorption spectrometer equipped with a Zeeman-effect background corrector was used as a comparative technique. An ultrasonic bath (Ultrasonic Cleaner 1400, Unique, São Paulo, Brazil) was used for degasification of samples. A Fluke 51 K/J thermometer with a K-type thermocouple, temperature range from -270 to +1370 °C, (Everett, WA, USA) was used for temperature measurements of the Ni tube.

2.2. Arrangement of the TS-FF-AAS system

The system consisted of a peristaltic pump with 8 channels (Ismatec, Labortechnik Analytik, Glattbrugg-Zürich, Switzerland) furnished with Tygon tubes, a lab-made commutator injector, and a lab-made thermospray flame furnace unit, which included the ceramic capillary (0.5 mm i.d., 2.0 mm e.d., and 100 mm of length) and the Ni tube (10 cm) which is located on the burner by a lab-made stainless steel support fixed with four ceramic pins. The tube was laid on these pins and could be moved into and out of the flame. For increasing the temperature inside the tube, six holes of 2 mm diameter were drilled in the bottom part of the tube. Another orifice was drilled at 90° to the bottom holes for inserting the thermospray capillary. The capillary tip was about 1 mm inside the tube furnace. The manifold was assembled with 0.5 mm i.d. PTFE tubing. A schematic diagram of the system can be seen in reference [14].

2.3. Reagents and samples

All solutions were prepared with analytical-reagent grade chemicals and ultrapure water obtained using a Milli-Q purifier system (Millipore, Bedford, MA, USA).

Reference solutions of metals were prepared by diluting the standard stock solutions containing 1000 mg l⁻¹ (Spex CertiPrep, Metuchen, NJ, USA) with deionized water.

Table 1
Instrumental parameters for Cu, Mn, Pb, and Zn measurements by FAAS

Parameter	Cu	Mn	Pb	Zn
Wavelength (nm)	324.7	279.5	217.0	213.9
Spectral band pass (nm)	0.5	0.2	1.0	1.0
Lamp current (mA)	4.0	5.0	10.0	5.0

Table 2
Graphite furnace heating program for Cu, Mn, Pb, and Zn determinations in beer samples

Stage	Temperature (°C)	Ramp (s)	Hold (s)	Ar flow-rate (ml min ⁻¹)
Drying I	90	5	30	3.0
Drying II	120	5	20	3.0
Pyrolysis	900 ^a , 800 ^b , 1100 ^c , 400 ^d	5	12 ^{a,b} , 10 ^c , 17 ^d	3.0
Atomization ^c	2300 ^a , 2400 ^b , 2600 ^c , 1900 ^d	2	10 ^{a,d} , 5 ^b , 2 ^c	0
Cleaning	2400 ^{a,b,d} , 2800 ^c	2	5 ^{a,b,d} , 2 ^c	3.0

^a Cu.

^b Mn.

^c Pb.

^d Zn.

^e Signal acquisition in this stage.

Acid diluted solutions containing 0.014 and 0.14 mol l⁻¹ HNO₃ solution were prepared by diluting concentrated nitric acid (Merck, Darmstadt, Germany). A 1000 mg l⁻¹ palladium solution (Spex Certiprep) was used as a chemical modifier for GFAAS determinations of all analytes.

Beer samples were obtained from a local store and represent some types of beer (Pilsner, non-alcoholic, and dark beers) readily available to consumers.

2.4. Procedure

The carrier fluid (0.14 mol l⁻¹ HNO₃ or air) and the height of the tube in relation to the burner head (0.3 or 1.5 cm) were optimized for each element. These parameters can affect the tube temperature and may potentially influence the atomization process. The internal diameter of the Ni tube is 10 mm. The analytical measurements were carried out at the minimum and maximum heights physically feasible. The tube temperature was measured at each height.

Beer samples were submitted to degasification in an ultrasonic bath for 20 min. Degasified samples were diluted in the range 1+1 to 1+5 v/v with 0.14 mol l⁻¹ HNO₃ depending on the analyte and the sample. These beer samples were spiked with different concentrations of metals and analyzed in the TS-FF-AAS system using optimized conditions.

For comparison of the results, all analytes were also determined by GFAAS after a 1+1 v/v dilution with 0.014 mol l⁻¹ HNO₃. The temperature program used for determination of Cu, Mn, Pb, and Zn is shown in Table 2. An aliquot of 5 µl of a solution containing 1000 µg ml⁻¹ Pd was added as chemical modifier in all determinations.

3. Results and discussion

The optimization was performed using solutions containing 100 µg l⁻¹ Cu, Mn, Pb, or Zn.

Two media were evaluated as carrier: diluted nitric acid and air. The evaluation of air as carrier can be justified because it avoids the pronounced dispersion typically observed at the interface between two liquids, as is the situation when diluted nitric acid is the carrier, and it does not cause any cooling effect of the Ni tube due to heat losses caused by solvent evaporation. Additionally, the absence of a liquid carrier decreases the dilution of the atomic cloud formed in the Ni tube. All these effects might cause an increment of sensitivity for all analytes. It could also be mentioned that one of the main practical limitations of the TS-FF-AAS system is that the temperature inside the Ni tube is not high enough to promote efficient atomization of less volatile elements. On the other hand, the use of air as carrier can cause memory effects because there is no permanent cleaning of the capillary as is the case when a solution is used as carrier. It was experimentally observed that lower memory effects were observed when diluted nitric acid was used as carrier.

The tube height is an important parameter because it can modify the tube temperature due to the temperature gradient in the flame. The maximum temperature is obtained in the flame at 1 cm above the primary zone of combustion [17]. The temperature at maximum (1.5 cm) and minimum (0.3 cm) height of the Ni tube was measured using a stoichiometric flame and air as carrier; the temperature inside the Ni tube was 1045 and 1090 °C for measurements at 0.3 cm and 1.5 cm, respectively.

The effects caused by observation height and carrier composition on absorbance signals are shown in Fig. 1. The absorbance signals for Cu and Mn did not vary appreciably for both carriers and observation heights tested. It is well known that Cu and Mn atomization in a flame is not severely affected by changing the experimental conditions for generation of the atomic cloud [17]. On the other hand, the highest signals for Pb and Zn were obtained by using a 0.14 mol l⁻¹ HNO₃ solution as carrier and minimum tube height. It can be seen in Fig. 1 that all differences are less

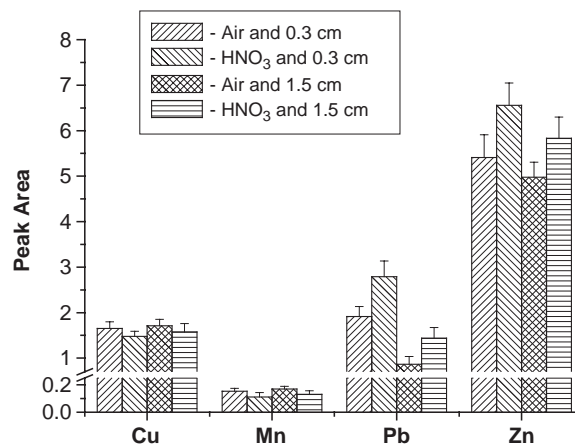


Fig. 1. Optimization of conditions for Cu, Mn, Pb, and Zn measurements by TS-FF-AAS: effects of carrier composition and Ni tube height on sensitivity.

than 25%, and this can be mainly related with the temperature variations, which are around 5%. Taking into account these results, all further measurements were carried out using nitric acid diluted solution as carrier and 0.3 cm as the observation height. The residence time was not measured, but it is long, as can be inferred by the duration of the transient signals. This is the most important feature of the Ni tube positioned in the flame, in addition to the 100% sample introduction efficiency.

The figures of merit for Cu and Zn are shown in Table 3. Calibration curves were obtained with aqueous reference solutions for all analytes. The limits of detection (LOD) and quantification (LOQ) were defined as the concentration corresponding to 3 and 10 times, respectively, the standard deviation of the blank ($n=10$) divided by the slope of the analytical curve. The limits of detection obtained for all elements in aqueous solution were appreciably lower than those attained by conventional FAAS also shown in Table 3 for comparison. The repeatability of 8 consecutive signals for beer samples diluted containing $50 \mu\text{g l}^{-1}$ of Cu and Mn and $25 \mu\text{g l}^{-1}$ of Pb and Zn was 3.5%, 5.6%, 7.3%, and 2.7%, respectively.

The concentrations of the metals were evaluated using the analyte addition technique, because calibration based on reference solutions prepared in aqueous medium led to negative errors for all analytes. Results were compared with those obtained by GFAAS. This technique was chosen for comparison because it presents proper sensitivity and because no reference material is available. The results obtained for Cu, Mn, Pb, and Zn are shown in Table 4.

According to a paired t -test, the results obtained by TS-FF-AAS based on the analyte addition technique and GFAAS are in agreement at a 95% confidence level. The analyte addition technique was efficient to correct for matrix effects observed when beer samples were introduced after simple dilution. This means that even without the pneumatic nebulization process, typical of conventional FAAS, the presence of an organic solvent at low concentration, around 5% for beer samples, and organic compounds, such as carbohydrates and proteins, still caused appreciable effects on the sample transport and/or the atomization process inside the Ni tube. In addition, the other tested procedure implied in a time-consuming evaporation step to minimize solvent effects.

Table 3
Analytical characteristics of the Cu, Mn, Pb, and Zn measurements by TS-FF-AAS

Parameter	Cu	Mn	Pb	Zn
Regression equation	$y=0.0064x-0.0352$	$y=0.0007x+0.0082$	$y=0.0183x+0.0023$	$y=0.1685x+1.0606$
R^2	0.9997	0.9870	0.9989	0.9963
Linear range ($\mu\text{g l}^{-1}$)	up to 400	up to 500	up to 200	up to 200
LOD (this work) ($\mu\text{g l}^{-1}$)	2.2	18	1.5	0.9
LOD (conventional FAAS) ($\mu\text{g l}^{-1}$) ^a	76	123	46	11
LOQ ($\mu\text{g l}^{-1}$)	7.3	59	5.2	3.1
Precision (%) ^b	3.5	5.6	7.3	2.7

^a Values obtained for conventional FAAS.

^b $n=8$.

Table 4

Metals determination in beer samples using TS-FF-AAS and GFAAS (mean \pm standard deviation, $\mu\text{g l}^{-1}$, $n=3$)

Sample	TS-FF-AAS (SAM)	GFAAS
<i>Cu</i>		
Pilsner 1	132 ± 5.4	129 ± 8.6
Pilsner 2	155 ± 7.3	160 ± 12.0
Non-alcoholic	108 ± 4.4	111 ± 7.3
Dark	38.0 ± 2.5	39.9 ± 3.1
<i>Mn</i>		
Pilsner 1	128 ± 3.6	133 ± 4.9
Pilsner 2	348 ± 13.3	355 ± 18.4
Non-alcoholic	224 ± 8.8	228 ± 10.6
Dark	110 ± 4.6	117 ± 6.0
<i>Pb</i>		
Pilsner 1	30.5 ± 2.9	29.0 ± 5.1
Pilsner 2	32.9 ± 4.3	34.6 ± 3.8
Non-alcoholic	13.0 ± 1.9	14.1 ± 1.2
Dark	19.7 ± 2.2	22.5 ± 1.8
<i>Zn</i>		
Pilsner 1	166 ± 2.3	168 ± 4.3
Pilsner 2	193 ± 5.4	189 ± 6.3
Non-alcoholic	226 ± 6.4	231 ± 5.7
Dark	52.7 ± 2.6	55.3 ± 3.9

Matusiewicz and Kopras [2] determined different elements in beer samples after acid digestion assisted by ultraviolet radiation, and Bellido-Milla et al. [11] after microwave-assisted digestion. In both works, the standard calibration technique and the analyte addition technique were compared and no differences were observed. This means that when the organic matter was eliminated by acid digestion, the matrix effect was minimized and the standard calibration technique led to accurate results. In the work described here, even when adopting 1:5 v/v sample dilution, matrix interfered in the determination.

The concentration range in different samples can vary greatly. The concentration of these elements depends of many factors: raw material quality, brewing process, environmental contamination, etc. Copper, Mn, and Pb concentrations determined are in agreement with the values found in the literature [1,2,12]. Zinc concentrations are slightly higher than those previously established for beer samples of other countries [12,18].

4. Conclusion

The TS-FF-AAS system has been used for the determination of trace metals in beer. The sensitivity obtained by TS-FF-AAS allowed direct analysis of beer samples prepared only by degasification and dilution. The analyte addition technique led to accurate results for Cu, Mn, Pb, and Zn in beer, whereas the standard calibration technique with reference solutions prepared in aqueous medium resulted in low recoveries for all elements due to matrix effects.

No digestion procedure was required, thus time and reagent consumption were minimized and safety was improved. Thermospray-FF-AAS presents lower cost and can be easily implemented. Additionally, TS-FF-AAS can expand the applicability of the widely available FAAS instruments. The proposed procedure is straightforward and results for Cu, Mn, Pb, and Zn can be quickly obtained for quality control in a brewery.

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